(a) is defective in that said adenovirus lacks the E1A and E1B transactivator sequences needed for its replication, but retains those sequences needed for said adenovirus to enter cells in which said adenovirus infects;

(b) comprises a set of sequences needed for encapsidation of said adenovirus; and

(c) comprises a nucleic acid sequence coding for a cytokine, wherein said nucleic acid sequence is under the control of a promoter of the rep gene of parvovirus Hl; and wherein said adenovirus vector also lacks the E3 region of the adenovirus.--

Entd 36

REMARKS

Entry of the foregoing and favorable reconsideration and reexamination of the subject application, as amended, pursuant to and consistent with 37 C.F.R. § 1.112, and in light of the remarks which follow are respectfully requested.

By the present amendment the specification has been amended to insert the proper headings. The specification has also been amended to add an Abstract page. The abstract is supported by the specification at page 1, line 27, to page 2, line 6. The specification has also been amended to add a Brief Description of the Figures. The support for this amendment can be found at page 11, lines 12-19. Applicants submit that these amendments to the specification do not represent new matter.

Claims 1 and 3 to 5 have been canceled. Applicants reserve their right to file a continued prosecution application or a request for continued examination directed to the canceled subject matter. Claims 6 to 8 have been amended and Claim 14 has been added. Support for the

promoter in Claim 14 can be found at least on page 14, line 15 of the application as filed. Applicants submit that no new matter has been added via this amendment.

Certified copy of the priority document

Applicants note that this case is a continuation of U. S. Application Ser. No. 08/619,157. The receipt of the certified copy of the priority document was acknowledged in an Office Action dated February 3, 1998 in that application. Please advise us of the status of the certified copy of the priority document.

35 U.S.C. § 112

Turning now to the Official Action, Claims 1 and 6 have been rejected under 35 U.S.C. § 112, second paragraph as being indefinite. These claims have been canceled. The newly submitted set of claims no longer recite the terms "capable of,", "carry genetic information," "essential sequences" "contains' or "containing." Therefore this rejection should now be rendered moot. Applicants respectfully request withdrawal of the rejection.

35 U.S.C. § 102(e)

Claims 1 and 3 to 8 have been rejected under 35 U.S.C. § 102(e) as being anticipated by Crystal, U.S. Patent No. 6,013,638. Claims 1 and 3 to 5 have been canceled. As far as this rejection may pertain to the claims now of record, this rejection is respectfully traversed.

Crystal et al describe pharmaceutical compositions and methods for adenoviral mediated transfer to the lung whereby desired proteins of interest are produced for local and/or systemic use therein.

Crystal et al fail to describe the selection and use of a promoter of the rep gene of parvovirus Hl in their adenoviral construct. This promoter permits a more specific expression of the cytokine which is restricted to tumor cells.Crystal et al do not disclose an adenoviral vector coding for cytokines and targeted for expression in tumor cells, thus requiring a specific promoter restricted to tumor cells. Hence, the presently claimed invention is not anticipated by Crystal et al.

Thus, in view of the above, withdrawal of this rejections is respectfully requested.

35 U.S.C. § 103(a)

Claims 1 and 3 to 8 have been rejected under 35 U.S.C. § 103(a) as being unpatentable over Rosenfeld et al taken with Russell and Ramshaw et al. Claims 1 and 3 to 5 have been canceled. As far as this rejection may pertain to the claims now of record, this rejection is respectfully traversed.

Rosenfeld et al teach an adenoviral vector which is deleted in the E3 region and **a portion of the viral E1a** coding region, while **retaining the E1b coding region**. The portion of the E1a coding region that was deleted was that portion that impairs viral replication.

Rosenfeld et al fail to teach or suggest deleting both the E1a and E1b transactivators; i.e., the entire E1 region. Nor does Rosenfeld et al teach or suggest transferring any other genes using their adenoviral vector other than those to treat hereditary disorders stemming from cystic fibrosis such as the α1-antitrypsin gene and the CF gene. Indeed, Rosenfeld et al is mainly concerned with the transfer of the α1-antitrypsin gene to the lung epithelium *in vivo*. Hence, there was no suggestion of expressing cytokines in the adenoviral vector disclosed in Rosenfeld et al.

Finally the promoter of the rep gene of parvovirus Hl is not taught nor suggested in Rosenfeld et al. This is not surprising since this reference is not at all concerned about treating tumors.

Applicants also submit that from the teachings of Russell the skilled artisan would not seek replication defective adenoviral vectors as currently claimed but rather replication competent viral vectors.

More specifically, Russell is a review article that discusses the various drawbacks with using lymphokine gene cell transfer protocols to treat cancer. The author reviews various different modes of gene delivery that may be beneficial in the future when using lymphokines to treat cancer and among these modes of delivery discussed are replication defective viral vectors, replication competent viral vectors or a combination of replication defective and replication competent viral vectors.

Furthermore, Russell teaches that the recombinant gene transfer vectors must be further targeted either by surface targeting or intracellular targeting. Thus, the surface targeting vectors would have to be further modified. For intracellular targeting specific viruses should be used as the vector and these viral vectors must have the ability to complete their life cycle in the intracellular environment of the tumor cells.

Moreover, Russell addresses the problems associated with using replication defective gene transfer vectors at page 197, second column where the following is stated:

> Recombinant gene transfer vectors may be replication defective, replication competent or a combination of the two. Although safer, the **disadvantages of replication defective vectors of this type are considerable**. The vectors must survive long enough *in vivo* to transfer their genes, be available in numbers equal to or greater than the number of target cells (10^{11}-10^{12}), be capable of penetrating even poorly vascularized tumor regions and of recognizing the correct target cell population (emphasis added).

Thus, the person skilled in the art would gather from the above teaching that there are enormous problems associated with using replication defective vectors.

Furthermore, Russell teaches away from using replication defective viral vectors and encourages the use of replication competent viral vectors. This is clear from the disclosure at page 198, first where the author clearly states:

> However, it is difficult to imagine how the problem of access to poorly vascularized tumor regions could be overcome except **by the use of replication competent viruses** either to carry the lymphokine gene or to provide helper functions to allow the spread of the defective vector (emphasis added).

Russell unambiguously describes an anti-tumor model using viruses which are therapeutically functional due to the fact that said viruses are still able to replicate.

The recombinant viruses disclosed in Russell which clearly have an effect on tumor cells (H1 or MVM viruses; see, page 199, 1st column) have the following characteristics:

1. The interleukin gene is inserted in place of a capsid coding sequence under the control of the p38 promoter leading to a "defective recombinant genome." This "defective" designation at page 199, line 22 of Russell does not refer to replicative deficiency but rather to the inability of the replicated genome to be encapsidated (since the virus lacks a capsid coding sequence it cannot express complete capsid as well). As disclosed at page 199, 2nd paragraph of Russell, the Parvovirus replication is controlled by the non-structural NS1 protein and not by the capsid protein. If a functional parallel could be established between adenoviruses and parvoviruses, one could compare on the one side, the E1 and NS1 regions, and on the other side, the E3 and capsid regions.
2. The recombinant viruses in Russell are able to replicate as described at page 199, lines 22-23. In contrast, in the present invention the defective recombinant

adenoviruses due to their deletion in E1 do not replicate in cells (see, at least page 3, 2nd paragraph of the specification).

3. In addition, the recombinant viruses in Russell are themselves cytotoxic to the transformed cells (see, page 199, lines 22-23) due to the fact that they express the NS1 cytotoxic protein (page 199, lines 11-12). Therefore, it is clear that the virus itself is responsible for cell death.

4. The recombinant viruses in Russell are improved in the sense that they further are able to express an interleukin. At page 199, lines 24-25 the following is stated: "which also induce interleukin production by the dying cell.."

The above passage clearly states that the cells are already killed when they express interleukin, confirming that the interleukin is not responsible for cell death in this viral model.

In conclusion, a gene therapy method disclosed by Russell is unambiguously associated with the use of recombinant viruses wherein the replication cycle is deeply associated with the desired cytotoxic anti-tumoral effect. This anti-tumoral effect is not only associated with interleukin, but with the expression by the parvovirus of the non-structural NS1 protein. This is confirmed by the third to last paragraph in Russell which indicates that interleukin expression plays a certain synergistic role which aims at improving the efficiency of the viral cytotoxic treatment which itself, does not ensure 100% efficiency.

In contrast, the defective adenoviral vectors described in the present invention are specifically chosen due to their non-cytotoxicity. Thus, the skilled artisan **when reviewing**

Russell as a whole, as required by law, would not adapt the defective adenoviral vectors for anti-cancer therapy of the tumor-cell-target-lymphokine gene therapy type.

Ramshaw et al disclose a recombinant vaccine which comprises a vaccine vector in which a first nucleotide sequence capable of being expressed as all or part of antigenic polypeptide together with a second nucleotide sequence capable of being expressed as all or part of a lymphokine. **The lymphokine is used to enhance the immune response to the antigenic polypeptide.** This fact is evidenced at least on pages 2 to 3 of this reference where the following is stated:

> The present invention is based on the recognition that the expression of appropriate lymphokines from recombinant bacterial or viral vaccines **can boost and/or modify the immune response** to viral, bacterial or co-expressed foreign antigenic polypeptides (emphasis added).

And again at page 8 of Ramshaw et al. where the following is stated:

> Although this invention has primarily been described with reference to vaccinia virus as the vaccine vector, it is to be **understood that the inventive concept resides in co-expression of an antigenic polypeptide and lymphokine**, ... (emphasis added)

Hence, it is clear from the teachings of Ramshaw et al that the lymphokine used in the vaccine vector acts **as an adjuvant** to the antigenic polypeptide; i.e., to increase the antigenicity of the antigenic polypeptide.

Furthermore, in Ramshaw, the E3 region was replaced with the interleukin gene. It is of importance to replace this region since the adjuvant qualities of the lymphokine are maintained. This is evidenced at page 14 of Ramshaw et al where the following is stated:

> The removal of the E3 region also prevents production of a virus protein that complexes with the major histocompatibility heavy chain protein and reduces the cell-mediated immune response to the virus.

Thus, when Ramshaw et al is viewed as a whole the skilled artisan would recognize the requirement of an antigenic polypeptide and a lymphokine to be used in the vaccine vector. A

skilled artisan would not be motivated to make a vaccine vector using a lymphokine alone since its purpose is that of an adjuvant to enhance immune response in a nondefective viral vector.

This adjuvant activity is especially important when the patients are suffering from AIDS, leprosy or cytomegalovirus infection since, as stated at page 6:

> ... co-expression of lymphokine could be instrumental in overcoming the defects to allow a normal response to the antigenic polypeptide and/or vector virus.

Hence, when read as a whole there is simply no teaching in Ramshaw et al that the lymphokine can be used alone in a vaccine vector.

Furthermore, Ramshaw et al describe various viruses that can be used to construct the vaccine vector. Among the viruses that one can choose from are poxvirus, herpes virus, adenovirus and vaccinia virus. No preferred choice as to the virus utilized in the vector is given, although vaccinia virus is referred to more often and is used in most of the examples. Therefore, a skilled artisan would not be motivated to choose an adenoviral vector over the other ones disclosed.

The Examiner refers only to the partial disclosure in the Abstract of Ramshaw et al stating that this reference discloses "the combination of adenoviral vector having lymphokine genes such as interleukin or gamma-interferon inserted therein (abstract)." However, in Example 4 of Ramshaw et al the construction of a recombinant **non-defective adenovirus**; i.e., **a viable adenovirus** in which the E3 region has been replaced by the interleukin gene is described. Thus, the adenoviral vectors described in Ramshaw et al would multiply in the cells that they invade, since the E1 region is maintained.

In contrast in the present invention discloses a defective adenoviral vector which does not multiply in the cells they invade as described. See, for example page 3, 2nd paragraph of the

specification. Thus, the amount of cytokine administered is controlled which is in contrast to the teachings of Ramshaw et al.

Thus, there is simply no teaching or suggestion in Ramshaw et al to use defective adenoviral vectors wherein the entire E1 region is lacking. Nor is there any teaching or suggestion in this reference of the specific promoter that is presently claimed.

The combination of references does not render the claimed invention obvious since the skilled artisan would not combine references encouraging and showing the use of competent adenoviral vectors as described in Russell and Ramshaw et al, respectively with the defective viral vector described in Rosenfeld et al. Indeed, even Russell makes a complete distinction between competent recombinant vectors and defective recombinant vectors. None of the cited prior art references either alone or in combination teach or even suggest that the E1 region of the adenoviral vector be totally deleted. Nor do any of these references suggest the use of the promoter from the rep gene of parvovirus Hl, which directs expression specifically in tumor cells.

Although Russell discloses the selection of promoters that would control the level of lymphokine secretion, they do not teach or suggest the use of promoters which direct expression specifically in tumor cells.

Therefore, in view of the above, withdrawal of this rejection is respectfully requested.

Claim 1 has been rejected under 35 U.S.C. § 103(a) as being unpatentable over Rosenfeld et al taken with Russell and Ramshaw et al., and further in view of Stratford-Perricaudet. Claim 1 has been canceled. As far as this rejection may pertain to the claims now of record, this rejection is respectfully traversed.

The combination of Rosenfeld et al taken with Russell and Ramshaw et al has been discussed extensively above. The arguments set forth therein are incorporated by reference into this rejection. It should be clear that none of these references teach the total deletion of the E1 region of a defective adenoviral vector and clearly suggest the use of said deleted defective adenoviral vector to insert a cytokine under the control of a promoter of the rep gene of parvovirus Hl.

The tertiary reference of Stratford-Perricaudet et al does not remedy the deficiencies of the primary reference. Stratford-Perricaudet et al reviews different attempts to adapt defective adenoviral vectors for use as a gene transfer vehicles to target various tissues and organs *in vivo*. More specifically, the nucleotide sequences inserted into the defective adenoviral vectors include a virus (HBsAg), an enzyme (orinithine transcarbamylase), β-galactosidase and α1-antitrypsin. These sequences were successfully targeted to specific tissues or organs such as the muscle, lung and liver in experimental animal models.

However, the use of these adenoviral vectors is somewhat questionable as evidenced in the final paragraph of D3, which states the following:

> If it proves appropriate to consider Adenovirus as a gene transfer vector for man, development of new adenovirus-based vectors, limiting the contribution of viral genes, will have to be undertaken.

Therefore, Stratford-Perricaudet et al teach the person skilled in the art that if adenoviral gene transfer vectors are to be used in man, then further adjustments have to be made to the adenoviral vector itself.

Moreover there is no appreciation whatsoever in Stratford-Perricaudet et al that tumor cells can be targeted and if targeted what type of insert in the adenoviral vector should be inserted that would reduce tumors. Furthermore, there is no disclosure nor suggestion of using a

promoter of the rep gene of parvovirus Hl that allows a more specific expression of the adenoviral vector in tumor cells.

Therefore in view of the above, withdrawal of this rejection is respectfully requested.

From the foregoing further and favorable action in the form of a Notice of Allowance is respectfully requested and earnestly solicited.

Respectfully submitted,

MERCHANT & GOULD P.C.
P. O. Box 2903
Minneapolis, Minnesota 55402-0903
(612) 332-5300

ATTORNEYS FOR APPLICANT(S)

Date Oct. 4, 2000

By Katherine M Kowalchyk

Katherine M. Kowalchyk
Reg. No. 36,848
(612) 371-5311